CLAIMS

1. A method of measuring oestrogen or progesterone receptor (ER or PR) status having the steps of:
   a) obtaining histopathological specimen image data; and
   b) identifying in the image data groups of contiguous pixels corresponding to respective cell nuclei;

   characterised in that the method also includes the steps of:
   c) deriving hue and saturation for the image data in a colour space having a hue coordinate and a saturation coordinate;
   d) thresholding the image data on the basis of hue and saturation and identifying pixels corresponding to cells which are preferentially stained relative to surrounding specimen tissue; and
   e) determining ER or PR status from proportion of pixels corresponding to preferentially stained cells.

2. A method of measuring ER or PR status having the steps of:
   a) obtaining histopathological specimen image data; and
   b) identifying in the image data groups of contiguous pixels corresponding to respective cell nuclei;

   characterised in that the method also includes the steps of:
   c) deriving hue and saturation for the image data in a colour space having a hue coordinate and a saturation coordinate;
   d) thresholding the image data on the basis of hue and saturation and identifying pixels corresponding to cells which are preferentially stained relative to surrounding specimen tissue; and
   e) determining ER or PR status from normalised average saturation.

3. A method of measuring ER or PR status having the steps of:
   a) obtaining histopathological specimen image data; and
   b) identifying in the image data groups of contiguous pixels corresponding to respective cell nuclei;

   characterised in that the method also includes the steps of:
   c) deriving hue and saturation for the image data in a colour space having a hue coordinate and a saturation coordinate;

d) thresholding the image data on the basis of hue and saturation and identifying pixels corresponding to cells which are preferentially stained relative to surrounding specimen tissue; and

e) determining ER or PR status from normalised average saturation and fraction of pixels corresponding to preferentially stained cells.

4. A method according to Claim 3 characterised in that step b) is implemented using a K-means clustering algorithm.

5. A method according to Claim 4 characterised in that the K-means clustering algorithm employs a Mahalanobis distance metric.

6. A method according to Claim 3 characterised in that step c) is implemented by transforming the image data into a chromaticity space, and deriving hue and saturation from image pixels and a reference colour.

7. A method according to Claim 6 characterised in that hue is obtained from an angle ϕ equal to $\sin^{-1} \frac{|\tilde{x}y - x\tilde{y}|}{\sqrt{\tilde{x}^2 + \tilde{y}^2}\sqrt{x^2 + y^2}}$ and saturation from an expression $\frac{x\tilde{x} + y\tilde{y}}{\tilde{x}^2 + \tilde{y}^2}$, where (x, y) and $(\tilde{x}, \tilde{y})$ are respectively image pixel coordinates and reference colour coordinates in the chromaticity space.

8. A method according to Claim 6 characterised in that hue is adapted to lie in the range 0 to 90 degrees and a hue threshold of 80 degrees is set in step d).

9. A method according to Claim 6 or 8 characterised in that a saturation threshold S_o is set in step d), S_o being 0.9 for saturation in the range 0:1 to 1.9 and 0 for saturation outside this range.

10. A method according to Claim 3 characterised in that the fraction of pixels corresponding to preferentially stained cells is determined by counting the number of pixels having both saturation greater than a saturation threshold and hue modulus less than a hue threshold and expressing such number as a fraction of a total number of pixels in the image.

11. A method according to Claim 3 characterised in that the normalised average saturation is accorded a score 0, 1, 2 or 3 according respectively to whether it is (i) ≤ 25%, (ii) > 25% and ≤ 50%, (iii) > 50% and ≤ 75% or (iv) > 75% and ≤ 100%.

12. A method according to Claim 11 characterised in that the fraction of pixels corresponding to preferentially stained cells is accorded a score 0, 1, 2, 3, 4 or 5 according respectively to whether it is (i) 0, (ii) > 0 and < 0.01, (iii) ≥ 0.01 and ≤ 0.10, (iv) ≥ 0.11 and ≤ 0.33, (v) ≥ 0.34 and ≤ 0.66 or (vi) ≥ 0.67 and ≤ 1.0.

13. A method according to Claim 12 characterised in that the scores for normalised average saturation and fraction of pixels corresponding to preferentially stained cells are added together to provide a measurement of ER or PR.

14. A method according to Claim 3 characterised in that the fraction of pixels corresponding to preferentially stained cells is accorded a score 0, 1, 2, 3, 4 or 5 according respectively to whether it is (i) 0, (ii) > 0 and < 0.01, (iii) ≥ 0.01 and ≤ 0.10, (iv) ≥ 0.11 and ≤ 0.33, (v) ≥ 0.34 and ≤ 0.66 or (vi) ≥ 0.67 and ≤ 1.0.

15. A method according to Claim 3 characterised in that step e) is carried out by obtaining a score for normalised average saturation and a score for fraction of pixels corresponding to preferentially stained cells and adding the scores together.

16. A method according to Claim 1, 2 or 3 characterised in that it also includes measuring C-erb-2 status by the following steps:

a) correlating window functions of different lengths with pixel sub-groups within the identified contiguous pixels groups to identify pixels associated with cell boundaries,

b) computing brightness-related measures of cell boundary brightness and sharpness and brightness extent around cell boundaries from pixels corresponding to cell boundaries,

c) comparing the brightness-related measures with predetermined equivalents obtained from comparison images associated with different values of C-erb-2, and

d) assigning to the image data a C-erb-2 value which is that associated with the comparison image having brightness-related measures closest to those determined for the image data.

17. A method according to Claim 1, 2, 3 or 16 characterised in that it also includes measuring vascularity by the following steps:

a) deriving hue and saturation for the image data in a colour space having a hue coordinate and a saturation coordinate;

b) producing a segmented image by thresholding the image data on the basis of hue and saturation;

c) identifying in the segmented image groups of contiguous pixels; and

d) determining vascularity from the total area of the groups of contiguous pixels which are sufficiently large to correspond to vascularity, such area being expressed as a proportion of the image data's total area.

18. A method of measuring C-erb-2 status having the steps of:

a) obtaining histopathological specimen image data; and

b) identifying in the image data contiguous pixel groups corresponding to respective cell nuclei associated with surrounding cell boundary staining;

characterised in that the method also includes the steps of:

c) correlating window functions of different lengths with pixel sub-groups within the identified contiguous pixels groups to identify pixels associated with cell boundaries,

d) computing brightness-related measures of cell boundary brightness and sharpness and brightness extent around cell boundaries from pixels corresponding to cell boundaries,

e) comparing the brightness-related measures with predetermined equivalents obtained from comparison images associated with different values of C-erb-2, and

f) assigning to the image data a C-erb-2 value which is that associated with the comparison image having brightness-related measures closest to those determined for the image data.

19. A method according to Claim 18 characterised in that at least some of the window functions have non-zero values of 6, 12, 24 and 48 pixels respectively and zero values elsewhere.

20. A method according to Claim 18 characterised in that pixels associated with a cell boundary are identified from a maximum correlation with a window function, the window function having a length which provides an estimate of cell boundary width.

21. A method according to Claim 18 characterised in that a brightness-related measure of cell boundary brightness and sharpness is computed in step d) using a calculation including dividing cell boundaries by their respective widths to provide normalised boundary magnitudes, selecting a fraction of the normalised boundary magnitudes each greater than unselected equivalents and summing the normalised boundary magnitudes of the selected fraction.

22. A method according to Claim 21 characterised in that in step d) a brightness-related measure of brightness extent around cell boundaries is computed using a calculation including dividing normalised boundary magnitudes into different magnitude groups each associated with a respective range of magnitudes, providing a respective magnitude sum of normalised boundary magnitudes for each magnitude group, and subtracting a smaller magnitude sum from a larger magnitude sum.

23. A method according to Claim 22 characterised in that the comparison image having brightness-related measures closest to those determined for the image data is determined from a Euclidean distance between the brightness-related measures of the comparison image and the image data.

24. A method according to Claim 18 characterised in that in step b) identifying in the image data contiguous pixel groups corresponding to respective cell nuclei is carried out by an adaptive thresholding technique arranged to maximise the number of contiguous pixel groups identified.

25. A method according to Claim 24 wherein the image data includes red, green and blue image planes characterised in that the adaptive thresholding technique includes:

a) generating a mean value μ_R and a standard deviation σ_R for pixels in the red image plane,

b) generating a cyan image plane from the image data and calculating a mean value μ_C for its pixels,

c) calculating a product $CMM\mu_C$ where CMM is a predetermined multiplier,

d) calculating a quantity R_B equal to the number of adjacent linear groups of pixels of predetermined length and including at least one cyan pixel which is less than $CMM\mu_C$,

e) for each red pixel calculating a threshold equal to $\{RMM\mu_R - \sigma_R(4 - R_B)\}$ and RMM is a predetermined multiplier,

f) forming a thresholded red image by discarding each red pixel that is greater than or equal to the threshold,

g) determining the number of contiguous pixel groups in the thresholded red image,

h) changing the values of RMM and CMM and iterating steps c) to g),

i) changing the values of RMM and CMM once more and iterating steps c) to g),

j) comparing the numbers of contiguous pixel groups determined in steps g) to i), treating the three pairs of values of RMM and CMM as points in a two dimensional space, selecting the pair of values of RMM and CMM associated with the lowest number of contiguous pixel groups, obtaining its reflection in the line joining the other two pairs of values of RMM and CMM, using this reflection as a new pair of values of RMM and CMM and iterating steps c) to g) and this step j).

26. A method according to Claim 25 characterised in that the first three pairs of RMM and CMM values referred to in step k) are 0.802 and 1.24, 0.903 and 0.903, and 1.24 and 0.802 respectively.

27. A method according to Claim 25 characterised in that that it includes prior to step g) removing brown pixels from the thresholded red image if like-located pixels in the cyan image are less than $CMM\mu_C$.

28. A method according to Claim 25 characterised in that it includes prior to step g) forming an edge-filtered cyan image, generating a standard deviation σ_C for its pixels and removing edge pixels from the thresholded red image if like-located pixels in the Sobel-filtered cyan image are greater than $(\mu_C + 1.5\sigma_C)$.

29. A method according to Claim 25 characterised in that it includes prior to step g) removing pixels corresponding to lipids from the thresholded red image if their red green and blue pixel values are all greater than the sum of the relevant colour's minimum value and 98% of its range of pixel values in each case.

30. A method according to Claim 25 characterised in that it includes prior to step g) subjecting the thresholded red image to a morphological closing operation.

31. A method of measuring vascularity having the steps of:

a) obtaining histopathological specimen image data;

characterised in that the method also includes the steps of:

b) deriving hue and saturation for the image data in a colour space having a hue coordinate and a saturation coordinate;

c) producing a segmented image by thresholding the image data on the basis of hue and saturation; and

d) identifying in the segmented image groups of contiguous pixels; and

e) determining vascularity from the total area of the groups of contiguous pixels which are sufficiently large to correspond to vascularity, such area being expressed as a proportion of the image data's total area.

32. A method according to Claim 31 wherein the image data comprises pixels with red, green and blue values designated R, G and B respectively, characterised in that a respective saturation value S is derived in step b) for each pixel by:

i) defining M and m for each pixel as respectively the maximum and minimum of R, G and B; and

ii) setting S to zero if m equals zero and setting S to (M - m)/M otherwise.

33. A method according to Claim 32 characterised in that hue values designated H are derived by:

a) defining new values newr, newg and newb for each pixel given by newr = (M – R)/(M – m), newg = (M – G)/(M – m) and newb = (M - B)/(M – m) in order to convert each pixel value into the difference between its magnitude and that of the maximum of the three colour magnitudes of that pixel, this difference being divided by the difference between the maximum and minimum of R, G and B, and

b) calculating H as tabulated immediately below:

M	H
0	180
R	60(newb – newg)*
G	60(2 + newr – newb)*
B	60(4 + newg – newr)*

* provided that if H proves to be >360, then 360 is subtracted from it, and if H proves to be <0, 360 is added to it.

34. A method according to Claim 33 characterised in that the step of producing a segmented image is implemented by designating for further processing only those pixels having both a hue H in the range 282-356 and a saturation S in the range 0.2 to 0.24.

35. A method according to Claim 34 characterised in that the step of identifying in the segmented image groups of contiguous pixels includes the step of spatially filtering such groups to remove groups having insufficient pixels to contribute to vascularity.

36. A method according to Claim 35 characterised in that the step of determining vascularity includes treating vascularity as having a high or a low value according to whether or not it is at least 31%.

37. A computer program for measuring ER or PR status incorporating program code for controlling computer apparatus to execute the steps of:

a) processing histopathological specimen image data to identify in the image data groups of contiguous pixels corresponding to respective cell nuclei;

characterised in that the program is also arranged to implement the steps of:

b) deriving hue and saturation for the image data in a colour space having a hue coordinate and a saturation coordinate;

c) thresholding the image data on the basis of hue and saturation and identifying pixels corresponding to cells which are preferentially stained relative to surrounding specimen tissue; and

d) determining ER or PR status from proportion of pixels corresponding to preferentially stained cells.

38. A computer program for measuring ER or PR status the program incorporating program code for controlling computer apparatus to execute the steps of:

a) processing histopathological specimen image data to identify in the image data groups of contiguous pixels corresponding to respective cell nuclei;

characterised in that the program is also arranged to implement the steps of:

b) deriving hue and saturation for the image data in a colour space having a hue coordinate and a saturation coordinate;

c) thresholding the image data on the basis of hue and saturation and identifying pixels corresponding to cells which are preferentially stained relative to surrounding specimen tissue; and

d) determining ER or PR status from normalised average saturation.

39. A computer program for measuring ER or PR status incorporating program code for controlling computer apparatus to execute the steps of:

a) processing histopathological specimen image data to identify in the image data groups of contiguous pixels corresponding to respective cell nuclei;

characterised in that the program is also arranged to implement the steps of:

b) deriving hue and saturation for the image data in a colour space having a hue coordinate and a saturation coordinate;

c) thresholding the image data on the basis of hue and saturation and identifying pixels corresponding to cells which are preferentially stained relative to surrounding specimen tissue; and

d) determining ER or PR status from normalised average saturation and fraction of pixels corresponding to preferentially stained cells.

40. A computer program for use in measuring C-erb-2 status incorporating program code for controlling computer apparatus to execute the steps of:

a) processing histopathological specimen image data to identify contiguous pixel groups corresponding to respective cell nuclei associated with surrounding cell boundary staining;

characterised in that the computer program is also arranged to implement the steps of:

b) correlating window functions of different lengths with pixel sub-groups within the identified contiguous pixels groups to identify pixels associated with cell boundaries,

c) computing brightness-related measures of cell boundary brightness and sharpness and brightness extent around cell boundaries from pixels corresponding to cell boundaries,

d) comparing the brightness-related measures with predetermined equivalents obtained from comparison images associated with different values of C-erb-2, and

e) assigning to the image data a C-erb-2 value which is that associated with the comparison image having brightness-related measures closest to those determined for the image data.

41. A computer program according to Claim 40 characterised in that at least some of the window functions have non-zero values of 6, 12, 24 and 48 pixels respectively and zero values elsewhere.

42. A computer program according to Claim 40 characterised in that pixels associated with a cell boundary are identified from a maximum correlation with a window function, the window function having a length which provides an estimate of cell boundary width.

43. A computer program according to Claim 40 characterised in that in step d) a brightness-related measure of cell boundary brightness and sharpness is computed using a calculation including dividing cell boundaries by their respective widths to provide normalised boundary magnitudes, selecting a fraction of the normalised boundary magnitudes each greater than unselected equivalents and summing the normalised boundary magnitudes of the selected fraction.

44. A computer program for use in measuring vascularity characterised in that it is arranged to control computer apparatus to execute the steps of:

a) using histopathological specimen image data to derive hue and saturation for the image data in a colour space having a hue coordinate and a saturation coordinate;

b) producing a segmented image by thresholding the image data on the basis of hue and saturation; and

c) identifying in the segmented image groups of contiguous pixels; and

d) determining vascularity from the total area of the groups of contiguous pixels which are sufficiently large to correspond to vascularity, such area being expressed as a proportion of the image data's total area.

45. A computer program according to Claim 44 wherein the image data comprises pixels with red, green and blue values designated R, G and B respectively, characterised in that a respective saturation value S is derived in step b) for each pixel by:

i) defining M and m for each pixel as respectively the maximum and minimum of R, G and B; and

ii) setting S to zero if m equals zero and setting S to (M - m)/M otherwise.

46. A computer program according to Claim 45 characterised in that hue values designated H are derived by:

a) defining new values newr, newg and newb for each pixel given by newr = (M – R)/(M – m), newg = (M – G)/(M – m) and newb = (M - B)/(M – m) in order to convert each pixel value into the difference between its magnitude and that of the maximum of the three colour magnitudes of that pixel, this difference being divided by the difference between the maximum and minimum of R, G and B, and

b) calculating H as tabulated immediately below:

M	H
0	180
R	60(newb – newg)*

G	60(2 + newr – newb)*
B	60(4 + newg – newr)*

* provided that if H proves to be >360, then 360 is subtracted from it, and if H proves to be <0, 360 is added to it.

47. Apparatus for measuring ER or PR status including means for photographing histopathological specimens to provide image data and computer apparatus to process the image data, the computer apparatus being programmed to identify in the image data groups of contiguous pixels corresponding to respective cell nuclei, characterised in that the computer apparatus is also programmed to execute the steps of:

a) deriving hue and saturation for the image data in a colour space having a hue coordinate and a saturation coordinate;

b) thresholding the image data on the basis of hue and saturation and identifying pixels corresponding to cells which are preferentially stained relative to surrounding specimen tissue; and

c) determining ER or PR status from proportion of pixels corresponding to preferentially stained cells.

48. Apparatus for measuring ER or PR status including means for photographing histopathological specimens to provide image data and computer apparatus to process the image data, the computer apparatus being programmed to identify in the image data groups of contiguous pixels corresponding to respective cell nuclei, characterised in that the computer apparatus is also programmed to execute the steps of:

a) deriving hue and saturation for the image data in a colour space having a hue coordinate and a saturation coordinate;

b) thresholding the image data on the basis of hue and saturation and identifying pixels corresponding to cells which are preferentially stained relative to surrounding specimen tissue; and

c) determining ER or PR status from normalised average saturation.

49. Apparatus for measuring ER or PR status including means for photographing histopathological specimens to provide image data and computer apparatus to process the image data, the computer apparatus being programmed to identify in

the image data groups of contiguous pixels corresponding to respective cell nuclei, characterised in that the computer apparatus is also programmed to execute the steps of:

a) deriving hue and saturation for the image data in a colour space having a hue coordinate and a saturation coordinate;

b) thresholding the image data on the basis of hue and saturation and identifying pixels corresponding to cells which are preferentially stained relative to surrounding specimen tissue; and

c) determining ER or PR status from normalised average saturation and fraction of pixels corresponding to preferentially stained cells.

50. Apparatus according to Claim 49 characterised in that it is programmed to implement step a) by transforming the image data into a chromaticity space, and deriving hue and saturation from image pixels and a reference colour.

51. Apparatus for measuring C-erb-2 status including means for photographing histopathological specimens to provide image data and computer apparatus to process the image data, the computer apparatus being programmed to identify in the image data groups of contiguous pixels corresponding to respective cell nuclei, characterised in that the computer apparatus is also programmed to execute the steps of:

a) correlating window functions of different lengths with pixel sub-groups within the identified contiguous pixels groups to identify pixels associated with cell boundaries,

b) computing brightness-related measures of cell boundary brightness and sharpness and brightness extent around cell boundaries from pixels corresponding to cell boundaries,

c) comparing the brightness-related measures with predetermined equivalents obtained from comparison images associated with different values of C-erb-2, and

d) assigning to the image data a C-erb-2 value which is that associated with the comparison image having brightness-related measures closest to those determined for the image data.

52. Apparatus according to Claim 51 characterised in that at least some of the window functions have non-zero values of 6, 12, 24 and 48 pixels respectively and zero values elsewhere.

53. Apparatus according to Claim 51 characterised in that the computer apparatus is programmed to identify pixels associated with a cell boundary from a maximum correlation with a window function, the window function having a length which provides an estimate of cell boundary width.

54. Apparatus according to Claim 51 characterised in that the computer apparatus is programmed to execute step b) by computing a brightness-related measure of cell boundary brightness and sharpness using a calculation including dividing cell boundaries by their respective widths to provide normalised boundary magnitudes, selecting a fraction of the normalised boundary magnitudes each greater than unselected equivalents and summing the normalised boundary magnitudes of the selected fraction.

55. Apparatus for measuring vascularity including means for photographing histopathological specimens to provide image data and computer apparatus to process the image data, characterised in that the computer apparatus is also programmed to execute the steps of:

a) deriving hue and saturation for the image data in a colour space having a hue coordinate and a saturation coordinate;

b) producing a segmented image by thresholding the image data on the basis of hue and saturation; and

c) identifying in the segmented image groups of contiguous pixels; and

d) determining vascularity from the total area of the groups of contiguous pixels which are sufficiently large to correspond to vascularity, such area being expressed as a proportion of the image data's total area.

56. Apparatus according to Claim 100 wherein the image data comprises pixels with red, green and blue values designated R, G and B respectively, characterised in that the computer apparatus is programmed to derive a respective saturation value S for each pixel in step b) by:

i) defining M and m for each pixel as respectively the maximum and minimum of R, G and B; and

ii) setting S to zero if m equals zero and setting S to (M - m)/M otherwise.